

POLYPNEUSTIC LOBES IN THE LARVAE
OF TSETSE-FLIES (*GLOSSINA*) AND
FOREST-FLIES (*HIPPOBOSCIDAE*)

BY

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Having occasion recently to determine the exact specific differences in the larvae of certain species of tsetse-flies, I discovered to my great joy that the innumerable 'papillae' which form the sculpturing on the exterior of the prominent lobes, at the anal extremity of the body, were respiratory openings, and evidently function as such during the intra-uterine life of the larva. In other words, each so-called 'papilla' represents a separate and distinct stigma, whose tubular continuations are connected with large tracheal trunks in the interior of the lobes. These compound stigmata appear as 'air-holes' of the simplest type, and when seen in optical section, under a low magnification ($\times 100$), their presence gives the integument the appearance, in miniature, of the 'rose' of a florist's watering-can. The ramifications of all the tracheal trunks which almost fill the interior of the lobes have not, owing to their complex nature, been completely traced to the main tracheal system, but there can, I think, be little doubt that they are all connected with the main pair of stigmata (posterior spiracles) which lie in the deep cup-shaped cavity or pit *between* the polypneustic lobes. This remarkable development of the respiratory system in the final larval stage can be seen only in those individuals which have been dissected out of the maternal uterus, or in examples which have been prematurely aborted. At the moment when the larva leaves the parent, or immediately prior to its birth, the compound stigmata seem to close up completely, the integument becomes intensely black, very strongly chitinised, and impervious to the

strongest beam of light. All that remains of the innumerable stigmata is their external form, the openings being, as far as one can trace, completely fused and obliterated by the closing up and hardening of the integument. On the other hand the sub-lying tracheal trunks remain more or less intact, and can be dissected out of the lobes even in the old effete larval skin which forms the puparium in the succeeding stage in the development of the insect. The paired and relatively large stigmata which lie in the cup-shaped pit remain open, but whether they also remain in vital connection with the nymph or pupa cannot be ascertained in the absence of fresh material. The large ligaments of the anal stigmata can, however, be seen projecting from the lobes into the puparium in well-preserved examples.

Austen (1903) in his description of the puparium ('pupa') says (p. 26) 'the posterior extremity (last segment) is produced on each side into a prominent tumid lip. These lips enclose a deep pit, within which in the larval state were situated the posterior stigmata or respiratory apertures.' He adds also in his more recent publication (1911), 'The tumid lips seen in the larva are equally conspicuous in the pupa, and the shape of the notch between them, in conjunction with the size and shape of the lips themselves affords a valuable means of identifying and distinguishing pupae belonging to different species.' He also figures the puparia of six different species.

Stuhlmann (1907) who gives a very good figure of the larva of '*Glossina fusca*' (= *G. brevipalpis*, Newst.), was of the opinion that the main tracheal system opens into the large cavity of the black, thickly chitinised end of the larva, but gives no details regarding the morphology of these structures.

Roubaud (1909), who ranks as one of the most famous students of the tsetse-flies, gives a very full description of the young larva with excellent drawings but no exact details regarding the true morphological structure of the anal lobes ('protubérances caudales') in the final larval stage. He suggests, however, that they function as a protection for the large paired stigmata which lie in the deep cup-shaped pit, thus forming a kind of air chamber favourable to gaseous exchange. He adds also (p. 464), 'Morphologiquement donc, les protubérances caudales, qui sont si caractéristiques des larves de

glossines, sont des appareils de protection des orifices respiratoires, développés aux dépens de la paroi postérieure du segment anal, par des évaginations latérales en ballonnets de l'hypoderme. Les deux orifices stigmatiques les plus externes de chaque côté, distendus et déformés par suite de ce mouvement, se sont trouvés, de plus, oblitérés par la pression des protubérances contre la paroi de l'utérus; ils ont cessé d'être fonctionnels, sacrifiés pour assurer le fonctionnement compensateur du troisième. Il y a donc là encore, un remarquable trait d'adaptation à la vie intra-utérine, spécial aux larves de glossines et qu'il est intéressant de mettre en évidence.'

The above are the more important papers on the subject, though the larvae of tsetse-flies have been referred to by various other authors since Sir David Bruce's (1895) remarkable discovery of the 'pupiparous' habit of these insects in 1895. So far, therefore, as I can trace, no student has hitherto dealt with the morphology of the anal lobes in the adult larva, and their true function has remained an unsolved problem until now.

Having determined the complicated and highly specialized character of the anal respiratory organs in *Glossina*, I proceeded at once to examine the larvae of some of the forest-flies (*Hippoboscidae*) whose anal segments are furnished with lobes of a similar character, though they are not nearly so prominent as those in the larvae of the tsetse-flies (*Glossina*). Having, in the first instance, selected a young adult larva of *Hippobosca maculata*, one of the commonest of the African forest-flies, I discovered that the low-convex anal lobes or callosities were distinctly polypneustic in character, and that the general form and structure of the stigmata and their tubular connections with the large tracheal trunks were very similar to those found in *Glossina*. The only marked difference was that the supernumerary stigmata in *Hippobosca maculata* were much fewer in number (about eighty on each lobe), and that they were arranged in three rather broad, bilateral bands all radiating from the larger paired stigmata which lie in the middle line between the lobes. The larva of the common forest-fly (*H. equina*) of this country has also a very similar arrangement, and so also has *Lynchia maura*.

In the larva of the 'sheep ked' (*Melophagus ovinus*) the respiratory system of the lobes is not nearly so complex and the

number of stigmata is reduced to four on each lobe in addition to the paired stigmata, so that collectively there are five pairs of stigmata. In this species *each lobe* is furnished with a deep cup-shaped pit, near the bottom of which, and occupying a sub-central position, is the anal stigmatic opening which communicates directly with the main abdominal air tubes (tracheae); near the rim or periphery of the pit are two large stigmata: one towards the venter the other towards the dorsum, in addition there is also a very minute pore-like stigma; and outside the pit an outer-lateral stigma rendered most conspicuous by its large and strongly chitinised peritreme. All the stigmata, with the possible exception of the very minute one, are connected together by a thick-walled air sac or trunk, which latter can be easily traced through the integument. Leuckart (1858), in his classical paper on this insect, describes the anomalous character of the stigmata in the adult larva, but in his description and figures (Table III, fig. 9) demonstrates that there are but three stigmata to each lobe; in this he was clearly in error, as there are undoubtedly five in all.

Massonnat (1909), in his extensive memoir on the pupipara, makes no reference to the function of the anal lobes in the larvae, but offers some interesting remarks on the general respiratory system of the larvae and adults. He states (pp. 174, 175) in regard to the latter that in the female the arrangement is a little different from that in the male. In the former there are to be seen an enormous mass of tracheae, which branch out over the uterus. They are divided into two groups: the first arising from the posterior stigmata, the others from the anterior abdominal and from the posterior thoracic stigmata. They all converge towards the middle of the abdomen and cover the uterus with their ramifications. This difference in the arrangement of the breathing organs in the female, he thinks, must certainly have relation to the mode of life of the larva and contribute to its respiration. He states furthermore that the tracheal system of the larva functions by means of the posterior stigmata, the air penetrating through the medium of the maternal vulva, but believes that it should be admitted that the lateral stigmata of the larva also take part in the respiratory interchange. In certain larvae he observed that the lateral (abdominal) stigmata were open, and that owing to the extraordinary abundance of

tracheae in the maternal uterus respiratory interchange between the larva and the mother must take place.

In *Glossina* there is also an abundance of tracheae in the region of the uterus; it is highly probable, therefore, that these organs function in a similar way and contribute to the respiration of the larva during its intra-uterine life, and also that the gaseous exchange is greatly facilitated by the presence of the polypneustic lobes. A study of serial sections will no doubt assist very materially in determining the inter-relationship of the respiratory system between the parent and offspring in both *Glossina* and the Pupipara.

CHARACTERS OF THE POLYPNEUSTIC LOBES

Glossina palpalis, Robineau-Desvoidy

(Figs. 1 and 2)

Polypneustic lobes. Stigmata (fig. 1, *c. st. 1.* profile) covering the whole exterior of the lobes in the form of low, nipple-like protuberances, arranged closely together so that their bases touch each other; collectively reminding one somewhat of the cast or mould of a seamstress's thimble, or individually of the 'nipple-like process' in the puparia of certain scale insects of the genus *Aspidiotus*. Each nipple-like structure has a central stigmatic opening (*c. st. 2.*), the rim of which is circular and very narrowly darker than the integument immediately surrounding it; in some of the openings there are faint traces of hair-like processes projecting into the lumen; these are few in number and are apparently analagous to the setae found in the stigmata of other insects; the thickenings of the integument forming the 'nipple' on the exterior show as two concentric rings; the first is narrow and very close to the opening; the second very broad and extending to the periphery. In many places there is a narrow, pale space between the structures which, when continuous, forms a faintly reticulated pattern.

Several attempts have been made to count the number of stigmata, but owing to their close proximity and regular disposition over the highly convex or rounded surface of the lobes no very definite figures have been obtained; but as all the counts which were made averaged over 500 to each lobe the total number may slightly exceed these figures.

The tubular continuation of each stigma is connected with large branching tracheal trunks (figs. 1, 2, *tr. tru.*), the latter extending across the interior from the outer to the inner lateral walls of the lobes so that they partly fill the interior. These vesicles have no annular thickenings or taenidia, but the walls, nevertheless, appear very rigid, and their surface presents a fine granular appearance

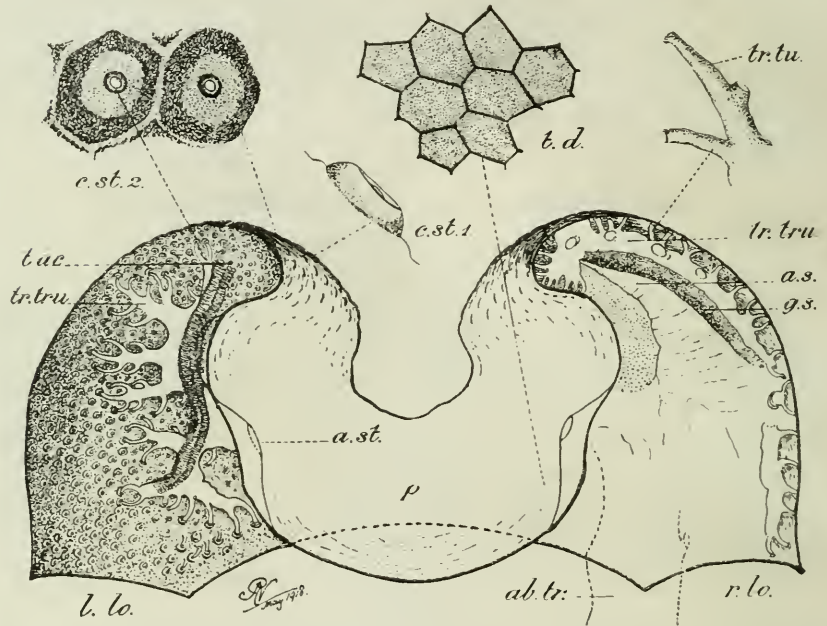


FIG. 1. *Glossina palpalis*: Polypneustic lobes of the young adult larva, seen in longitudinal section in the plane immediately above the paired abdominal stigmata (*a. st.*); *l. lo.*, left lobe; *r. lo.*, right lobe; *p.*, cup-shaped pit between the lobes; *c. st. 2*, compound stigmata as seen by transmitted light; *c. st. 1*, one of the compound stigma as seen in profile; *t. d.*, tessellated dermal cells of the integument, forming the wall of the pit; *tr. tru.*, tracheal trunks with their lateral branches leading to the stigmata in the wall of the lobe; *tae.*, double tracheal tube with incomplete taenidia; *a. s.*, air sac; *g. s.*, granular portion of air sac; *ab. tr.*, relative position of main tracheal tube leading to the abdominal segments.

as if faintly pigmented. In the middle line opposite the region of the terminal, paired abdominal stigmata (fig. 1, *a. st.*) is a large, thin-walled air-sac (fig. 1, *a. s.*), which though ruptured in my preparations seems to be directly connected with the former; it is finely striated transversely on its inner lateral portion and granulated towards the periphery of the lobe (fig. 1, *g. s.*) On

either side of the sac is a double tracheal tube (*tae.*) with apparently incomplete or somewhat irregular taenidia; it lies in the long axis of the lobe, but is imperfect in my preparations, so that its exact course cannot be traced. So far as one can judge, the whole of this very complicated respiratory system of the lobes is connected in some way with the main paired stigmata, but this requires verification, which a further study may give.

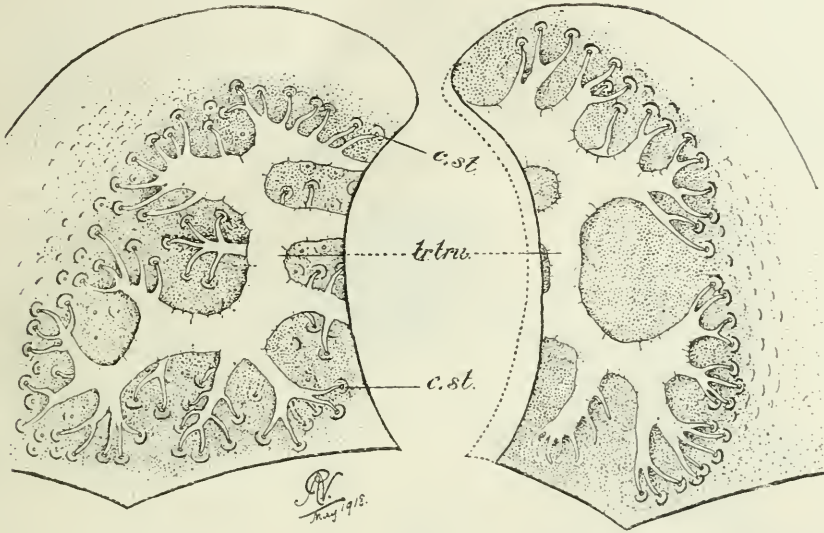


FIG. 2. *Glossina palpalis*: Polypneustic lobes of young adult larva, more highly magnified than in fig. 1, and the cup-shaped pit omitted. Interior, showing tracheal trunks and compound stigmata in two different planes.

The foregoing description is based upon a single larva dissected from the uterus of the parent.* The body was divided longitudinally along the medio-lateral line and placed in cold KOH for a few hours and finally mounted in Canada balsam with the cut surfaces uppermost so as to get a clear view of the internal structures of the lobes.

Glossina ? fusca

(Fig. 3)

The following details are based upon fragments of the lobes which were broken up, in the first instance, in order to study the

* Captured by Dr. J. Schwetz, Belgian Congo, 1914, to whom I am much indebted for extensive collections of these insects.

integumental character. No very exact particulars of the respiratory system can therefore be given, but I may add that it was the examination of these dissections that led to the discovery of the remarkable respiratory character of the lobes. The larva in question was collected by the late Dr. J. Everett Dutton and Dr. J. L. Todd during their expedition to the Congo Free State in 1904, unfortunately I cannot give its exact specific determination, but it is probably referable to *G. fusca*, Walker. There can, however, be no doubt that it belongs to the 'Fusca Group' of tsetse-flies as defined by me in the *Bulletin of Entomological Research* (1911); beyond this it is not safe to go, as several closely-allied species occur in the Congo Free State whose larvae await discovery.

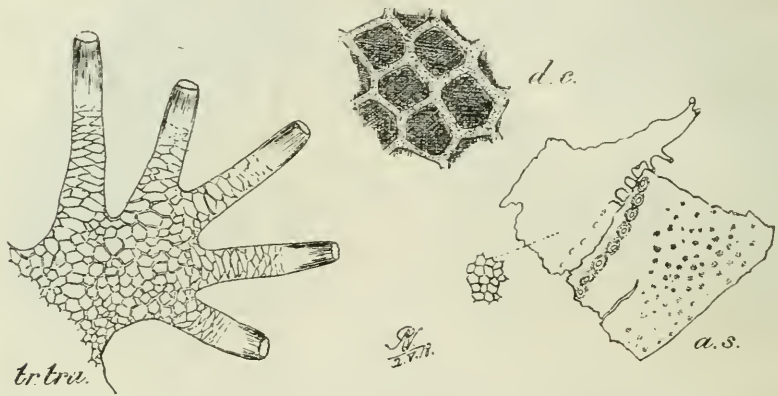


FIG. 3. *Glossina ? fusca*: *d. c.*, tessellated dermal cells of abdomen of larva (from a stained preparation); *tr. tru.*, distal or terminal portion of tracheal trunk with digit-like lateral tubes connected with the stigmata; *a. s.*, air sac, showing the lateral tubes connected with a row of stigmata.

Polypneustic lobes. Stigmata circular or ovate arranged, more or less in groups or loop-like series numbering from fourteen to sixteen in each; in places also in definite straight lines or single rows (fig. 3, *a. s.*), the respective rows being widely separated. These 'loop-like' groups are connected with relatively short, stout tubes (tracheae) which branch off from the main trunk, so that when these structures are dissected out with the distal end of the tracheal trunk intact they appear distinctly dactyliform in character (fig. 3, *tr. tru.*). The walls of the tracheal trunks and tubes are strongly and

irregularly reticulated, with the exception of the distal end of the lateral connective tubes at their junction with the stigmata. The tubes connected with the well-defined rows of stigmata (fig. 3, *a. s.*) are similar in form to the others, but they branch off from a relatively very large stiff-walled air-sac (fig. 3, *a. s.*) whose dimensions and exact form I have not been able to determine.

Glossina morsitans, Westwood

Three old adult larvae of this species, which were bred from captive flies by Dr. J. B. Davey and myself in Nyasaland in 1910, were dissected. The microscopical examination of these have given very disappointing results, as owing to the density of the chitin I have not been able to discover a trace of the open compound stigmata, even with a beam of strong sunlight passing through the condenser of the microscope. But portions of the main tracheal trunks with their lateral, connective, stigmatic tubes were found. The arrangement of the stigmata, as indicated externally, appear, however, to be very similar to those in *G. palpalis*.

Glossina ? morsitans, Westwood

(Fig. 4)

I have also examined a larva bearing the label '*Glossina morsitans*,' but without further data; and although the sculpturing of the lobes was very similar to that in *Glossina morsitans*, Westwood, I am not at all certain that the specimen in question is referable to this species or not. From this example were obtained some relatively large fragments of the tracheal trunks with a few of the lateral tubes intact (fig. 4); but all of these, though treated with KOH in the same way as the others, have remained quite opaque, blackish in colour, very rigid, and almost as hard and brittle as the chitinous walls of the lobes. Whether similar conditions obtain in other species of tsetse-flies I am unable to state. No tracheal organs with such dense and highly chitinised walls as these have been found in the lobes of the puparia of any other specimens of

tsetse-fly hitherto examined. It is possible that the tracheal tubes may harden and thicken simultaneously with the closing of the compound stigmata, but this one cannot at present verify; I therefore record the fact for future reference.



FIG. 4. *Glossina ? morsitans*: Fragments of the highly chitinised tracheal organs dissected from the polypneustic lobes of the larva.

PUPIPARA (FOREST-FLIES)

Hippobosca maculata, Leach

(Fig. 5)

Polypneustic lobes. These, although of considerable dimensions, form but low, convex protuberances with a very shallow depression between them, in which the single pair of abdominal stigmata (fig. 5, *a. st.*) and the micropylar process are placed near the centre. The shallowness of the lobes is, however, of very distinct advantage, as it enables one to examine them in optical section and thereby follow the ramifications of the sub-lying tracheal system with comparative ease, providing always that the integument is sufficiently transparent; such conditions obtain only in young adult larvae dissected from the uterus, as in *Glossina*. Old adult larvae with the dense black chitinous walls to the lobes are useless for an exact study of the respiratory system. The compound stigmata (fig. 5, *c. st.*) are arranged in three broad and well-defined tracks, each series diverging from a point very near to the main anal

stigmata (fig. 5, *a. st.*), and branching bilaterally towards the periphery of the lobe; the openings are small and pore-like and surrounded by three or more concentric rings of darker chitin; the

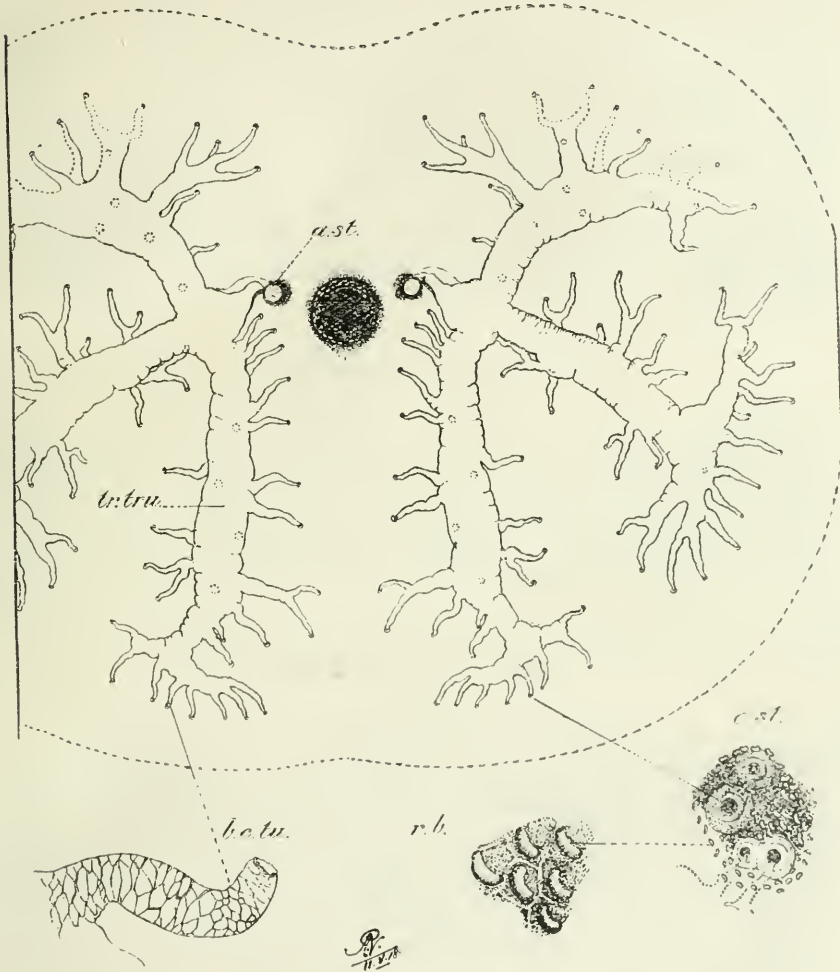


FIG. 5. *Hippobosca maculata*: Polypneustic lobes of the young adult larva; *a. st.*, anal stigmata; *c. st.*, compound stigmata; *r. b.*, reniform thickenings of the body-wall; *l. c. tu.*, lateral tube connecting main tracheal trunk with the stigma; *tr. tru.*, tracheal trunk.

spaces between the stigmata rather thickly studded with narrowly reniform thickenings of the body wall (fig. 5, *r. b.*). The tracheal trunks (fig. 5, *tr. tru.*) consist of three large irregularly cylindrical

tubes, so arranged that they lie immediately below the stigmatic openings; these main tubes give off small lateral tubes which for the most part are single, but a few of the longer ones are bifurcated, and each lateral tube is connected with a single stigma. The walls of the tracheal trunks, together with their lateral connective tubes, seem to be relatively thick and rigid, and the surface is strongly but irregularly reticulated. All the main trunks are united proximally, and connected with the main abdominal stigmata by a short commissure, which is really a continuation of the tracheal trunk much attenuated at its union with the paired stigmata. The individual tracheal trunks present a very remarkable appearance, reminding one somewhat of a grotesquely formed or distorted scolopendrid.

Hippobosca equina

Polypneustic lobes. Very similar in form to those in *H. maculata* and with similarly arranged supernumerary stigmata and tracheal trunks. The details regarding these structures in this species are being worked out, and a description of them will appear in further communications on this subject. New Forest, 1906. (R. Newstead.)

Lynchia maura, Bigot

(Fig. 6)

Polypneustic lobes with three large bilateral marginal extensions, those on either side of the anus somewhat angular, the others broadly rounded and strongly produced; margin of all extensions strongly undulating or wavy. Anal stigmata occupying a sub-central position with the micropylar process between them. The supernumerary stigmata are relatively small, and number about forty-six on either side; the connective tubes are relatively long, but fairly robust. The tracheal trunks relatively narrow, and consisting of three very widely divergent branches, each branch supplying the stigmata along the wavy margin with lateral connective tubes; the dorsal and ventral branches (*d. l. tru.* and *v. tr. tru.*) have each a backwardly directed branch whose terminal stigmata lie very near to the anal stigmata.

Owing to several large fractures in my preparation I cannot trace the commissure of the tracheal trunks with the main stigmata, but

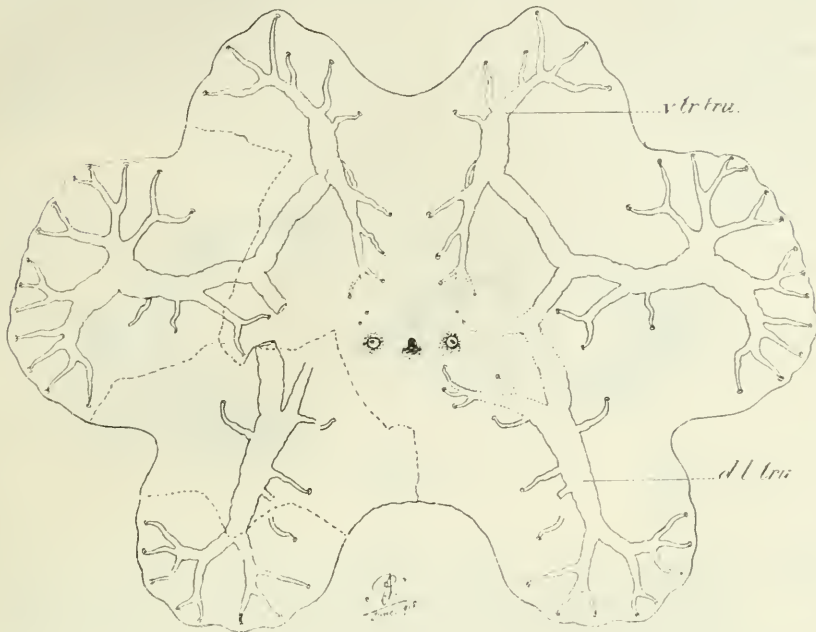


FIG. 6. *Lynchia maura*: Polypneustic lobes of young adult larva; *d. l. tru.*, dorsal tracheal trunk; *v. tr. tru.*, ventral tracheal trunk. Right side transposed, dotted lines = lines of restoration.

I have indicated its probable course by dotted lines as it is possible that the union of the tracheal trunks with the abdominal tracheal system may be similar to that in *Hippobosca maculata* (fig. 5).

Melophagus ovinus

(Fig. 7)

Polypneustic lobes. The lobes in this species are quite rudimentary though very clearly defined. Near the centre of each lobe is a large, deep, circular and cup-shaped depression; *inside* this pit or open chamber is placed, near the lower portion of the wall, the well-defined main abdominal stigma (*a. st.*), rendered most conspicuous by its clear circular opening; near the edge of the pit

are two slightly larger stigmata (*st. 1.* and *st. 2.*), and one very minute one (*st. 3.*); on the outer lateral side of the pit is another large stigma (*o. lat. st.*), rendered most conspicuous by its large, heavily chitinised peritreme. All the four large stigmata are connected together by a stiff-walled air sac (*a. sc.*) having a finely reticulated surface. Further details of the anatomy of these organs will be given when serial sections have been studied.

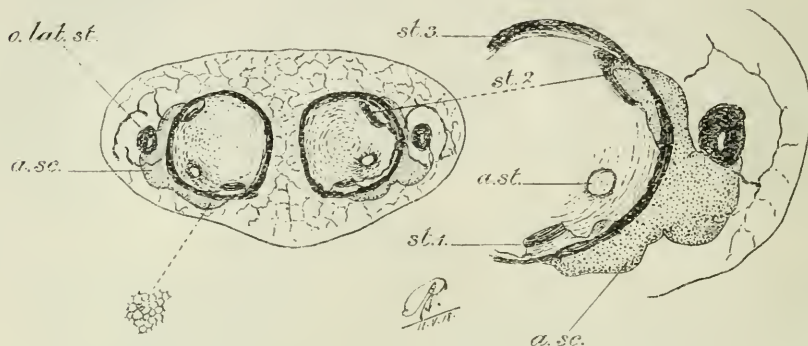


FIG. 7. *Melophagus ovinus*: Polypneustic lobes of young adult larva; *a. st.*, one of the paired abdominal stigmata; *st. 1-st. 3.*, supernumerary stigmata; *o. lat. st.*, outer lateral stigmata; *a. sc.*, connective air sac or trunk.

CONCLUSIONS

This paper is intended only as a preliminary one, as a further study of the polypneustic lobes of the Glossinae and Hippoboscidae is in progress. There are also other puparia of the *Diptera Cyclorhapha* possessing similar anal appendages which need investigation, notably several species found in Africa in similar situations to those of *Glossina*: some, in fact, were found in association with *G. morsitans* in Nyasaland (Newstead and Davy). Whether these appendages are in any way homologous* with those in *Glossina* remains to be seen; a study of them may, however, throw some light on the systematic position of the tsetse-flies. For the moment, however,

* Two distinct species have now been found to possess polypneustic lobes; but the generic position of these has, so far, not been determined.

it would seem that *Glossina* exhibits very composite characters with a distinct leaning towards the Hippoboscidae. In fact, one may go further and say that the larvae of the two groups, respectively, are so similar in form and structure that, if studied apart from the imagines, one would not hesitate to group them together as members of the same family.

N.B.—The figures of the polypneustic lobes, with the exception of those in fig. 2, are drawn to the same scale.

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